



Arsonic acid-presenting superparamagnetic iron oxide for pH-responsive aggregation under slightly acidic conditions

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ABSTRACT

We report the synthesis and characterization of the arsonic acid-presenting superparamagnetic iron oxide (SPIO). We used arsonoacetic acid as the ligand for SPIOs in aqueous media. The surface modification of the SPIOs was accomplished via the ligand exchange from undecanoic acid to the carboxyl moiety of arsonoacetic acid. Consequently, the well-dispersed arsonic acid-presenting SPIOs in water were obtained. We found that the dispersion state of the arsonic acid-presenting SPIOs can be sharply regulated by pH changes in the biological significant region. The well dispersion state of the arsonic acid-presenting SPIOs can be maintained at the neutral pH region. In contrast, the arsonic acid-presenting SPIOs can sensitively form the aggregation below pH 6.1. Moreover, these dispersion states can be controlled reversibly by the pH alteration in the narrow region.

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1. Introduction

Since the magnetism of SPIOs (superparamagnetic iron oxides) can be enhanced by the assembly, resulting in the creation of negative contrasts in the image, various kinds of the surface modifications which can change the dispersibility of SPIOs by the biological molecules and reactions have been synthesized.^{1,2} Indeed, the monitoring for the significant biological events have been successfully accomplished using the surface-modified SPIOs for the applications as biosensors.³ In particular, the SPIOs which can form the aggregation under slightly acidic condition are promised to be a tagging agent for tumor tissues because it is known that the pH values of the tumor tissues are from 0.4 to 1.0 units lower than those of the normal tissues (pH 7.4).⁴

The pH-responsiveness of the metal nanoparticles is dominated by the dissociation constants of the acid groups of the surfactants.⁵ For example, Toshima and co-workers reported that the carboxylic acid-modified gold particles can form the aggregation below pH 3.8.^{5a} Fiurasek and Reven synthesized phosphonic acid-modified gold particles which can gather below pH 5.5.^{5b} Arsonic acid has higher acid dissociation constant ($pK_{a1} = 4.5$, $pK_{a2} = 9.0$) than those of carboxylic acid ($pK_a = 4.0$) or phosphoric acid ($pK_{a1} = 2.0$, $pK_{a2} = 7.5$).⁶ Hence, arsonic acid derivatives can be expected to be new series of the surface modifications for providing the particles

with the ability to change the dispersion state under mild acidic conditions.

Herein, we report the synthesis and characterization of the arsonic acid-presenting SPIOs. The synthesized SPIOs were well-dispersed in aqueous media, and the assembly of the arsonic acid-presenting SPIOs occurs below pH 6.1. In addition, the assembly and dispersion of the arsonic acid-presenting SPIOs can sharply and reversibly proceed corresponding to the pH changes with borderline at pH 6.1. This is the first report not only about the arsonic acid-presenting metal nanoparticles but also about the feasibility of arsonic acid as a pH-responsive unit for pH-sensing probes.

2. Experimental section

2.1. General

¹H NMR spectra were obtained with a JEOL EX-400 spectrometer (400 MHz). Transmission electron microscopy (TEM) was performed using a JEOL JEM-100SX operated at 100 kV electron beam acceleration voltage. One drop of the sample solution was deposited onto a copper grid and the excess of the droplet was blotted off the grids with filter paper; then the sample was dried under ambient conditions. Powder X-ray diffraction (XRD) patterns were recorded on a SHIMADZU X-ray diffractometer-6000 with high-intensity Cu K α radiation at a scanning rate of 0.02° s⁻¹ in 2 θ ranging from 2° to 90°. FT-IR spectra were recorded on a Perkin Elmer 1600 infrared spectrophotometer using a KBr disk dispersed with the powder sample. X-ray fluorescence (XRF) spectra were recorded on a Rigaku Primini with PdK α radiation ($\lambda = 0.05859$ nm).

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Magnetization measurements were performed at room temperature using MPMS-XL5R SQUID susceptometer, Quantum Design Co. Ltd. The powder samples were used for XRD, FT-IR, XRF, and SQUID measurements. Dynamic light scattering (DLS) was measured to determine the hydrodynamic radii (r_H) of the samples on an FPAR-1000, Otsuka electronics Co. Ltd.

2.2. Materials

Iron(II) chloride hexahydrate, dithiothreitol and sodium hydride were purchased from Wako Pure Chemical Industries, Ltd (Osaka, Japan). Iron(III) chloride tetrahydrate, was purchased from Kanto Chemicals Co. Ltd (Tokyo, Japan). All reagents were used as supplied, unless stated otherwise. Arsonoacetic acid (**1**) was synthesized according to the previous work.⁷

2.3. Synthesis of the SPIOs

Preparation of the SPIOs was according to our previous report.⁸ Iron(III) chloride hexahydrate (1.081 g, 4 mmol) and iron(II) chloride tetrahydrate (0.3976 g, 2 mmol) were dissolved in water (120 mL). After the addition of oleic acid (0.2 mL) with mechanically stirring at 1000 rpm, 15 mL of aqueous ammonium hydroxylate (28%) was added to the solution all at once. Undecanoic acid (0.2 mL) was continuously added to the solution in four additions every 5 min with stirring at 1000 rpm at 80 °C. After stirring for 30 min, the resulting dark brown suspension was cooled to room temperature.

2.4. Ligand exchange

Undecanoic acid-coated SPIOs (10 mg) were dispersed in toluene (2 mL), and the ligand **1** (27 mg) as a suspension in methanol (1 mL) was dropwised under sonication. The dispersion was continuously sonicated overnight. The arsonic acid-presenting SPIOs were collected and washed with water for five times using a magnet.

2.5. Determination of the relaxivities

All samples (100 μ L) containing the modified SPIOs in the deionized water were sealed into 5 mm diameter of glass tubes. For the MR imaging and the calculation of transverse (T_2) relaxa-

tion times, the samples were added to the same volume of the Agarsec XP (Nippon gene) solution at the concentration of 1 wt %. MR imaging of the samples was carried out using a 7 T Unity Inova MR Scanner (Varian, Palo Alto, CA). Coronal images of the samples were obtained with a T_2 -weighted spin-echo sequence. (Repetition time (TR) 3000 ms, echo time (TE) 100 ms, the field of view was 40 mm, with an image matrix of 256×256 . Slice thickness was 5 mm.)

2.6. Aggregation and dispersion of the SPIOs by changing pH

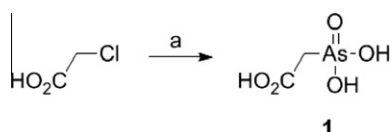
To adjust the pH of the samples containing 50 μ g/mL of the SPIOs, concd. hydrochloric acid (10 N) and 13 M sodium hydroxide were added. The DLS measurements were carried out at 25 °C. The CONTIN program was used for data analysis to determine the average hydrodynamic size.

3. Results and discussion

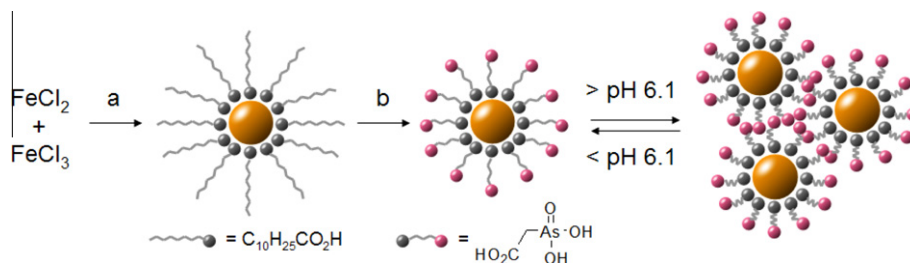
As illustrated in Scheme 1, we initially synthesized arsonoacetic acid (**1**) as the stabilizer for the SPIOs from chloroacetic acid and arsenic trioxide in 13 M NaOH aqueous solution.⁷ The chemical structure of the ligand **1** was characterized by ¹H NMR, ¹³C NMR, and FT-IR. The undecanoic acid-covered SPIOs were prepared according to the previous reports.⁸ According to Scheme 2, the **1**-covered SPIO particles were prepared by a ligand exchange from the undecanoic acid ligand. As shown in Figure 1, the SPIOs before the ligand exchange were well-dispersed in CHCl₃. In contrast, after the treatment with the ligand **1**, the resulting SPIOs showed good dispersibility only in water. This fact suggests that the ligand exchange on the surface of the SPIOs proceeded. The averages of r_H values of the SPIOs before and after the ligand exchange measured by DLS were determined as 9.1 ± 3.5 nm and 9.4 ± 2.4 nm, respectively. These results indicate that nonspecific aggregation of the particles should hardly occur.

The ligand **1** has the arsonic and carboxylic acid moieties, which are potentially able to form the stable bonds to the surface of SPIOs. To determine which functional groups between the carboxylic and arsonic acid are used for the co-ordination to the surface of the particles, FT-IR measurements were performed. Figure 2 shows the spectra of the ligands and the SPIOs. The significant peaks assigned as asymmetrical stretching vibration of C=O were observed around 1600 cm⁻¹ in both the spectra of the ligand **1** and **1**-covered SPIOs.⁹ The difference of the peak positions was +11 cm⁻¹. In contrast, there was a slight difference in the peak positions of the band derived from As–O and As=O stretching vibration (-1 cm⁻¹).¹⁰ These results strongly suggest that the ligand **1** should largely co-ordinate to the particles at the carboxylic unit, but not at the arsonic acid unit. A hard base like carboxylate anion might be favorable to form higher stable co-ordination with the iron oxide surface.¹¹

Elemental analysis and XRF spectroscopy suggested that the number of the arsonic acid presented from the single SPIO was



Scheme 1. Synthesis and the chemical structure of the ligand used in this study according to the Ref. 7. Reagents and conditions: (a) As₂O₃, water, 25 °C, 1 h.



Scheme 2. Synthesis and pH-dependent assembly of the arsonic acid-presenting SPIOs. Reagents and conditions: (a) undecanoic acid, ammonium hydroxide, water, 70 °C, 1 h; (b) **1**, toluene, methanol, sonication for 30 min and then stored at rt for 16 h.

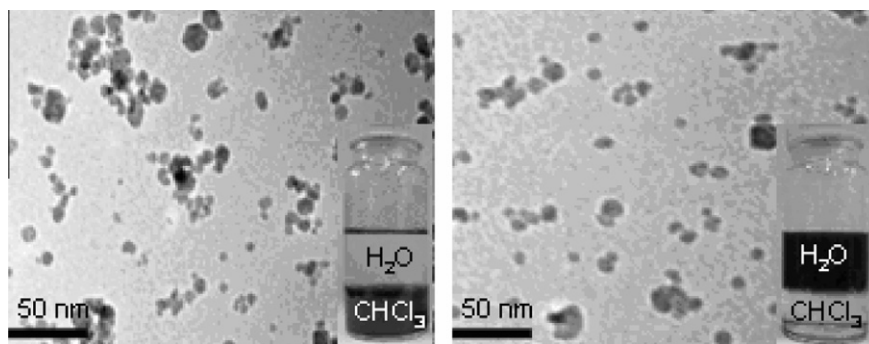


Figure 1. TEM images of the synthetic SPIOs before (left) and after (right) the ligand exchange.

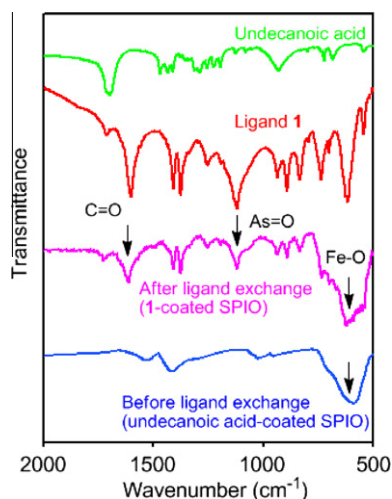


Figure 2. FT-IR spectra of the ligands and the SPIOs.

approximately 600. It has been reported that the interfacial areas of carboxylate are between 30 and 40 Å².¹² From these facts, it can be roughly estimated that the surface of the particles could be fully covered by the ligand **1**. This estimation represents that the ligand exchange proceeded quantitatively.

Figure 3 shows the results of XRD measurements of SPIOs before and after the ligand exchange. According to the similar peak patterns from the samples before and after the ligand exchange, it was confirmed that both SPIOs involve the spinel structure which is typical for magnetite.⁸ The crystallite size was also estimated by the half-height width of the strongest reflection (3 1 1)

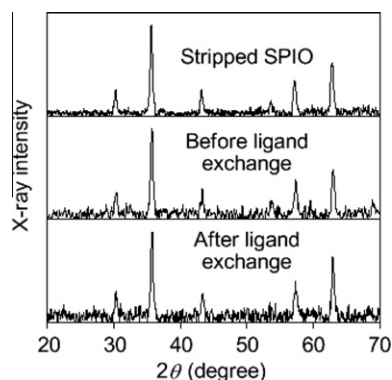


Figure 3. X-ray diffraction patterns of the SPIOs before (upper) and after (lower) the ligand exchange.

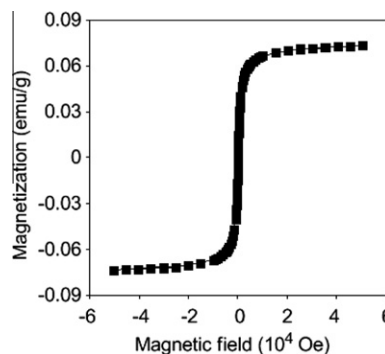


Figure 4. Magnetic hysteresis of the arsonic acid-presenting SPIOs.

plane, using Scherrer formula. The sizes of the undecanoic acid-, and arsonic acid-presenting SPIOs were estimated as 9.6 nm and 9.8 nm, respectively. These values represent good agreements with those of the r_H values. These results suggest that the ligand exchange occurred only at the surface of the particles and less affected to the magnetic property of the particle cores.

To confirm the superparamagnetism of the particles, the magnetization behaviors of the particles were investigated with the superconducting quantum interference device. As shown in Figure 4, the magnetization curves which represent strong magnetization (saturation magnetization is 73.1 memu/g) were obtained from the arsonic acid-presenting SPIOs. In addition, magnetization can proceed reversibly without magnetic hysteresis at room temperature. These facts clearly indicate that the particles have superparamagnetism.⁸ Since SPIOs can be representatively used as a negative contrast agent in MRI due to the significant ability for enhancing T_2 relaxation in water tissues, their relaxivities were calculated from the T_2 -dependency on the concentration. The samples containing the SPIOs dispersed in deionized water with various concentrations (5, 10, and 25 µg/mL) were sealed into 5 mm diameter of glass tubes, and T_2 -weighted MRI was performed at 7 T. The T_2 relaxation time was determined with a T_2 -mapping mode, and the relaxivity was calculated from the slopes of the lines according to Figure 5. The arsonic acid-presenting SPIOs provided approximately 2.5-times larger relaxivity (3.4 mL µg⁻¹ s⁻¹) than those of Resovist® (1.3 mL µg⁻¹ s⁻¹)⁸ which is the commercial contrast agent. The thin modification layer on the particles could less disturb the magnetic interaction of the SPIO core with water tissues.^{3d}

To examine the pH dependency on the assembly of the arsonic acid-presenting SPIOs, the r_H values were monitored at variable pH (Fig. 6). The pH titration was performed by adding hydrochloric acid to the solution. The SPIOs were well-dispersed in the aqueous media at pH 9. While the r_H values observed from the dispersions

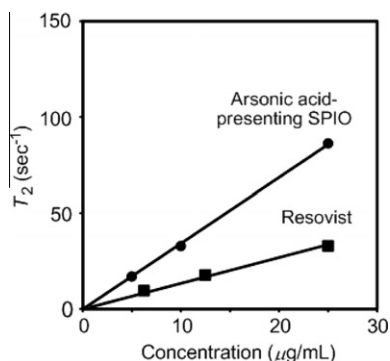


Figure 5. T_2 dependency on the SPIO concentration. The SPIOs were dispersed in deionized water, and T_2 values were measured at 25 °C. The relaxivities were determined by the slope of the linear approximation.

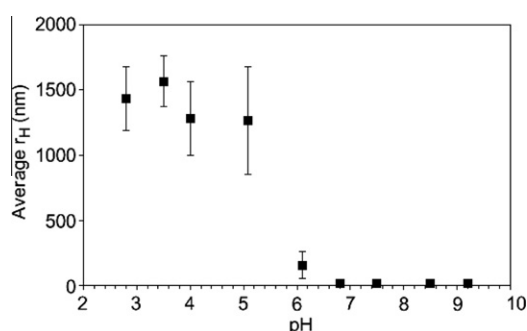


Figure 6. The pH-dependent aggregation of the arsonic acid-presenting SPIOs.

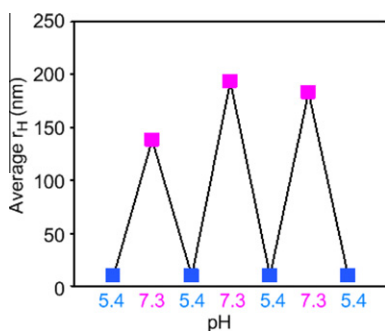


Figure 7. Reversibility of pH-responsive assembly of the arsonic acid-presenting SPIOs.

were below 50 nm in the pH range from 7 to 9, the r_H value of the surfactant significantly increased at pH 6.1 with the formation of the precipitation. Subsequently, the r_H values reached a plateau below pH 6. These results indicate that the arsonic acid-presenting SPIOs can assemble with borderline at pH 6.1. In addition, the assembly/dispersion of the SPIOs can proceed reversibly through several pH changes (Fig. 7). The acid dissociation constants (pK_a)

of aliphatic arsonic acids are $pK_{a1} = 4.5$, $pK_{a2} = 9.0$ in the solution.⁶ The aggregation could be due to protonation of arsonic acid unit resulting in decrease of static electronic repulsion and increase of hydrogen bonds between the SPIOs (Fig. 8). Precipitation or color changes were less observed during the pH titration. Moreover, average diameters of the particles hardly changed from the pristine dispersion. These data suggest that the arsonic acid unit can be stably immobilized on the surface of the SPIOs in this pH region.

4. Conclusion

In summary, we described the synthesis and characterization of the arsonic acid-presenting SPIOs. We demonstrated that the aggregation and dispersion states of the particles can be reversibly converted corresponding to the pH changes with borderline at pH 6.1. It has been known that the active tumor region tends to be slightly acidic. The aggregation of SPIOs can generate clear contrast in MRI. Hence, our materials can be expected to be applied as a good marker for the detection of the cancer cells with MRI.

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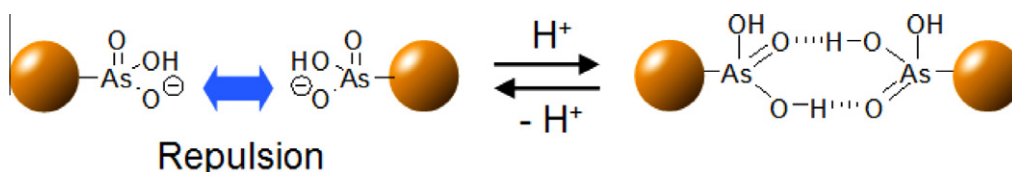


Figure 8. Proposed model of the pH-sensitive dispersion and assembly of the arsonic acid-presenting SPIOs.

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